

EFFECTS OF *Zingiberofficinale* AND SEEDS OF *Aframomummelegueta* EXTRACT ON SOME BIOCHEMICAL AND IMMUNOLOGICAL INDICES OF ELECTRIC FOOT SHOCK STRESS-INDUCED WISTAR RATS

ABSTRACT

Stress in general captures the process that individuals experience when environmental demands exceed the capacity of an individual, there is consistent evidence that stress is associated with a variety of negative health outcomes which happens through a variety of mechanisms. The purpose of this study was to investigate the effects of *Zingiberofficinale* and the seeds of *Aframomummelegueta* extracts on some biochemical and immunological indices of electric foot shock stress-induced Wistar rats. Forty-five Wistar rats were randomized into nine groups (A-I) of five rats per group. Group A served as standard control and was not induced with electric shock, group B served as negative control hence they were induced and untreated, group C served as the positive control and was administered 2.9 mg/kg magnesium while groups D and E were administered 100 and 200 mg/kg body weight of *Zingiberofficinale* extract and groups F and G were administered 100 and 200 mg/kg body weight of *Aframomummelegueta* extract respectively, while co extract of *Z. officinale* and *A. melegueta* was administered to group H and I at a dose of 100 and 200 mg/kg body weight respectively. At the end of the experimental period, the animals were sacrificed and the sera obtained were used for bioassay analysis. There was a significant decrease ($P < 0.05$) in the cortisol levels of extract-treated groups from week 1 (2.56 ± 0.55 $\mu\text{g/dl}$) to week 4 (1.85 ± 0.30 $\mu\text{g/dl}$) compared to the untreated group (3.98 ± 0.69 $\mu\text{g/dl}$) (week 1) and (8.57 ± 1.31 $\mu\text{g/dl}$) (week 4) as well as the kidney function test. The liver function test revealed a significant decrease in liver biomarkers (Alanine Aminotransferase, Aspartate Aminotransferase, Alkaline Phosphatase, direct bilirubin, and total bilirubin) in the extract-treated groups compared to the untreated control group. The extract of the plants impacted the White blood cell and its differentials while there was a significant decrease in the C-reactive protein in the extract-treated group (2.38 ± 0.22 mg/l) compared to the untreated control (9.93 ± 1.50 mg/l). The findings from this research showed that the extracts of *Z. officinale* and *A. melegueta* affects some biochemical and immunological parameters and hence could serve as a potential therapeutic agent to fight against stress and its related disorders.

Keywords: *Aframomummelegueta*, C- reactive protein, electric footshock, kidney function, liver function, stress, *Zingiberofficinale*,

INTRODUCTION

“Stress is considered to be physical and psychological tension resulting from an adverse situation or environment to which an individual struggle to adapt and maintain homeostasis” [1]. “According to the type and severity of the stimulus, stress can not only disrupt homeostasis but may also cause various diseases and can even prove fatal” [2]. “Stress is associated with diseases, such as ischemic stroke, cardiovascular disease, inflammatory bowel disease, and atopic dermatitis as well with psychiatric disorders” [3]. “The hypothalamic-pituitary-adrenocortical axis is an important neuroendocrine system that moderates responses to psychological and physical stress and inflammation” [4]. “Stress represents the main environmental risk factor for mental illness. Exposure to stressful events particularly early in life has been associated with increased incidence and susceptibility of major depressive disorders as well as other psychiatric illnesses” [4]. “Among the key players in these events are glucocorticoid receptors. Dysfunctional glucocorticoid signaling may indeed contribute to psychopathology through a number of mechanisms that regulate the response to acute or chronic stress and that affect the function of genes and systems known to be relevant for mood disorders” [5].

“Electric foot shocks have been widely used for the development of various animal models of human disorders such as hypertension, anxiety, depression, and post-traumatic stress disorder (PTSD) by introducing subtle variations in current intensity, duration, number of shock exposures, and post-exposure treatment” [6]. “In the animal model of stress-induced hypertension, rats received intermittent electric shocks, but behavioral changes of rats exposed to this chronic foot shock stress (CFSS) paradigm and nutritional treatment to manage the level of stress induced is been explored in this work”. [6]

“Overproduction of free radicals, such as reactive oxygen species (ROS) in the body plays an important part in the development of many chronic diseases. A variety of natural products possess antioxidant potential, such as vegetables, fruits, edible flowers, cereal grains, medicinal plants, and herbal infusions” [7]. Traditionally many plants have been used successfully for medicinal purposes [8].

A great number of the world's population; particularly Nigerians, rely on traditional medicines “for their primary healthcare needs” [9]. “These medicinal plants contain substances used for therapeutic purposes or are precursors for the synthesis of useful drugs” [9, 10]. “Ginger also has high antioxidant activity” [8]. “The rhizomes of ginger are the most widely used spice and condiment” [11]. “The consumption of ginger has been claimed to be useful in many oxidative stress-related medical conditions because of its anti-inflammatory effects, some of these medical conditions include hypertension, and diabetes-induced pancreatic and renal derangements” [12].

“Several medicinal importance has been attributed to ginger and alligator pepper plants; they include their therapeutic properties against a wide range of infections and health conditions such as cancer, cold, fever, coughs, nausea, arthritis, Alzheimer's disease, inflammations, and rheumatism” [13]. “Gingerol, one of the constituents of *Zingiber officinale* has been shown to be useful in treating inflammatory diseases such as arthritis and asthma” [13]. “Over the years, ginger has been highly regarded in the health and wellness product market as an economically important herb and their demands have continued to rise in many parts of the world” [14].

“*Aframomum melegueta* (Alligator pepper) also known as grains of paradise, is a tropical perennial herb of the genus *Aframomum* and the family, *Zingiberaceae* also known as the ginger family” [15]. Alligator pepper is greatly utilized by many countries including Nigeria for diverse purposes, *Aframomum melegueta* is a cosmopolitan plant employed in the Federal Republic of

Nigeria as a spice in foods. It is locally called ‘atare’ in Yoruba and ‘ose-oji’ in Igbo [16]. “It is a common item used for traditional sacrifices and other religious rites. Also, several uses of alligator pepper have been reported in the literature and these include its efficacy in controlling Egyptian cotton leaf worm and also important crop pests such as the diamondback moth” [17]. “Alligator pepper has immense medicinal importance; the seed extract of alligator pepper heals wounds and invigorates the immune system against diseases” [17]. “Studies have shown that seeds contain important phytochemicals namely, alkaloids, glycosides, tannins, flavonoids, sterols, triterpenes, and oils, some of which are responsible for their antimicrobial properties” [18].

There is no defined cure for stress, though there appear to be a drugs that have been used to manage it but these drugs are usually costly and could cause adverse effects, hence the need for plant-based remedies with reduced or no adverse effects. This study, therefore investigated the effects of *Zingiberofficinale* and seeds of *Aframomummelegueta* extract on the biochemical and immunological indices of electric foot shock stress-induced rats.

MATERIALS AND METHODS

Sample Collection and Identification

The rhizomes of *Zingiberofficinale* and *Aframomummelegueta* were purchased from Eke Awka market in Awka South Local Government Area of Anambra state. The samples were identified and authenticated by a Botanist in the Department of Botany, NnamdiAzikiwe University Awka, Anambra State. The voucher number for *Zingiberofficinale* deposited in the herbarium of NnamdiAzikiwe University Awkais NAUH-26A (Rhizome) while that of *Aframomummelegueta* is NAUH-13^A.

Preparation of Ethanol extracts of *Zingiberofficinale* and *Aframomummelegueta* Sample

Fresh *Zingiberofficinale* rhizomes were thoroughly washed, peeled, and chopped while the seeds of *Aframomummelegueta* were carefully selected and washed, both samples were air-dried at room temperature for four weeks and then ground into powder using Corona manual grinding machine.

Exactly 500g of the pulverized dried *Zingiberofficinale* and *Aframomummelegueta* seeds were macerated in 2 Litres of 70% ethanol. The ethanol extraction was allowed to stand for 48 hours for complete extraction after which it was separated, sieved using sieve cloth (Muslin cloth), and filtered using Whatman filter paper. The filtrate was separated and concentrated using a water bath at 500°C [19].

ANIMAL STUDIES AND GROUPINGS

A total of 45 Male Wistar Albino rats with 200g- 250g/body weights and 18 months of age were procured from Chris Animal Farm and Research Laboratory MgbakwuAwka, Anambra State and used for the experiment. They were maintained and housed in cages at the Chris Animal Farm and Research Laboratory MgbakwuAwka, according to the Institutional Animal Care and Use Committee (IACUC) guidelines on the care and handling of experimental animals. The animals were allowed to acclimatize for 7 days, and fed *ad libitum* (i.e the diet and water was available at all times) with vital grower’s mash pellets purchased from Vital Feed Distributor at Awka,

Anambra state. At the end of the 7 days' acclimatization period, the animals were weighed and then randomized into 9 groups of 5 rats each. Group A was the normal control, group B was the stressed without treatment, group C was stressed and treated with standard drug (2.9 mg/kg body weight magnesium supplement) calculated using the anesthesiology clinics guide 2013 issue for 21 days, groups D, E, groups F, G and groups H, I were stressed and treated with 100 mg/kg and 200mg/kg body weight because the result of the mean lethal dose(LD₅₀) shows that the best dose to use were 100mg/kg and 200mg/kg of the ethanol leaf extract of *Z. officinale*, *A. melegueta* and the combination of both *Z. officinale*, *A. melegueta* at 1:1 respectively for a period of 21 days after the induction of electric shock- induced stress to check the ability of the extracts in preventing or managing stress. Stress was induced using the electric shock maze at 110volts, 0.2 seconds shock was induced four times to the rats at an interval of 5 minutes daily. Bodyweights, Cortisol levels of the rats was determined before initiating the daily treatment with 100mg/kg and 200mg/kg of the ethanol extracts of *Z. officinale* and *A. melegueta* for a period of 21 days. After 28 day of treatment, the animals were sacrificed and a total number of 45 blood samples were collected for biochemical and immunological analysis.

ACUTE TOXICITY (LD₅₀) EVALUATION

The median lethal dose (LD₅₀) for *Zingiber officinale* and *Aframomum melegueta* was determined using [20].

INDUCTION OF ACUTE STRESS BY ELECTRIC FOOT SHOCK

Stress was induced using the electric-foot shock maze/ chamber at 110volts. The induction was done four times daily at 5minutes interval for 28 days, after the induction Treatment was done daily immediately after induction for 28days.



Figure 1: Stress induction process

Photographed by: Nwarienne. M. Chiamaka, 2022

ANIMAL SACRIFICE AND SAMPLE COLLECTION

After 28 days of stress induction and treatment, the animals were anaesthetized with chloroform and blood samples collected via cardiac puncture. The samples were collected into the universal bottles and allowed to clot, after which they were centrifuged for 10 minutes at 4000 rpm. The

serum obtained was transferred into another set of test tubes and was used for the biochemical and immunological estimations.

BIOCHEMICAL ASSAY

DETERMINATION OF CORTISOL LEVELS

Their Cortisol levels were checked at about 8.00am in the morning before the induction, during the process of induction and after the completion of the treatment of the stressed animals. It was measured using a Cortisol ELISA Kit (Fine Test, Wuhan, China) and Corticosterone ELISA Kit (Enzo Life Sciences, Ann Arbor, MI, USA) according to the manufacturer's instructions.

DETERMINATION OF KIDNEY FUNCTION TEST

Urea and creatinine was analysed using the Limdi and Hyde method [21].

DETERMINATION OF LIVER FUNCTION TEST

The liver function test was conducted using the method of Limdi and Hyde method [21].

DETERMINATION OF WHITE BLOOD DIFFERENTIALS

The haematological parameters were carried out using 5-part Auto Haematology Analyzer (BC-5300)

DETERMINATION OF C-REACTIVE PROTEIN

The determination of C- reactive protein was carried out using an essential reagent required for an immune enzyme metric assay which include high affinity and specificity antibodies (enzyme and immobilized) with different and distinct epitope recognition in excess and native antigen, as described by [22].

DATA ANALYSIS

The results were analyzed using the IBM SPSS version 25 (SPSS Inc., Chicago, Illinois, USA). All the numerical values were expressed as Mean \pm SEM. Statistical analysis of the results obtained was performed by using ANOVA Tests to determine if a significant difference exists between the mean of the test and control groups. The level of significance was set at $p < 0.05$.

QUANTIFICATION BY GC- FID

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. Phytochemical were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals expressed in ug/g [23].

RESULTS

RESULTS FOR CORTISOL LEVELS

Figure 2 shows the effect of ethanol extract of *Z. officinale* and *A. melegueta* on the Cortisol level of electric shock-stressed induced rats before, during, and after the treatment period. The result showed a significant decrease ($P < 0.05$) in the cortisol levels of extract-treated groups compared with the untreated control, with co-administration of *Z. officinale* and *A. melegueta* at 200 mg/kg body weight bringing the cortisol level to nearly zero.

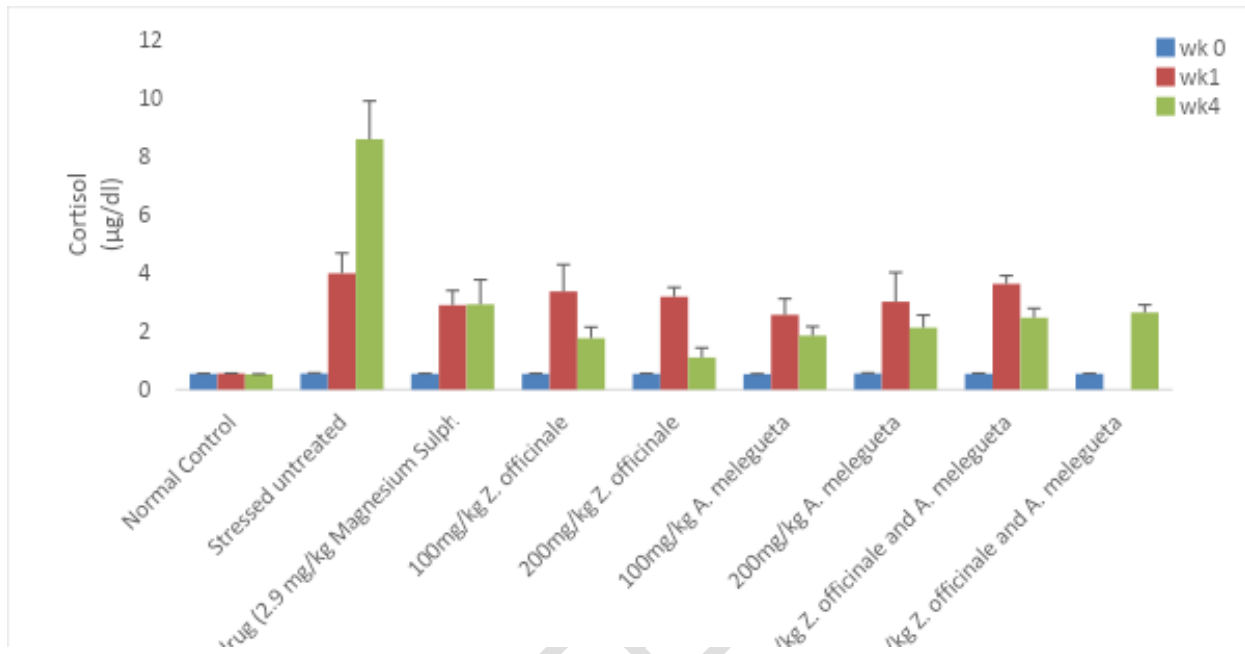


Figure 2: Shows the effect of ethanol extract of *Zingiber officinale* and *Aframomum melegueta* on Cortisol level of electric-shock stressed-induced rats before, during and after treatment period

RESULT FOR KIDNEY FUNCTION TEST

The result of the kidney function test is presented in Table 1, it showed a significant decrease ($P<0.05$) in urea and creatinine levels of the extracts treated groups compared with the untreated control.

Table 1: Effects of ethanol extract of *Z. officinale* and *A. melegueta* on Urea and Creatinine concentration of Electric- shock stress induced rats.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
Group A: Normal Control	0.48 ± 0.06	0.17 ± 0.01
Group B: Stressed untreated	0.69 ± 0.12	0.19 ± 0.01
Group C: Standard drug (2.9 mg/kg Magnesium Sulphate)	0.66 ± 0.12a	0.17 ± 0.01b
Group D: 100mg/kg <i>Z. officinale</i>	0.61 ± 0.07a	0.17 ± 0.01b
Group E: 200mg/kg <i>Z. officinale</i>	0.63 ± 0.13a	0.18 ± 0.02b
Group F: 100mg/kg <i>A. melegueta</i>	0.41 ± 0.07a	0.18 ± 0.01b
Group G: 200mg/kg <i>A. melegueta</i>	0.60 ± 0.11a	0.17 ± 0.01b
Group H: 100mg/kg <i>Z. officinale</i> + <i>A. melegueta</i>	0.51 ± 0.10a	0.19 ± 0.05b
Group I: 200mg/kg <i>Z. officinale</i> + <i>A. melegueta</i>	0.49 ± 0.11a	0.18 ± 0.01b

Note: a total of 45 wistar rats were used for this analysis

a=Significant decrease ($P<0.05$) in urea levels with respect to group B (stressed untreated group); b=Significant decrease ($P<0.05$) in creatinine levels with respect to group B (Stressed untreated group)

RESULTS FOR LIVER FUNCTION TEST

Table 2 shows the effect of ethanol extract of *Zingiber officinale* and *Aframomum melegueta* on liver function tests in electric-shock stress-induced rats. The result showed a significant decrease ($P < 0.05$) in the activities of ALT, AST, ALP, direct bilirubin, and total bilirubin in the extract-treated groups compared with the untreated control.

Table 2: Effects of ethanol extract of *Z. officinale* and *A. melegueta* on Liver Function Test in Electric- shock stress induced rats.

Groups	Liver Function Test						
	ALT (U/L)	AST (U/L))	ALP (U/L)	D. (mg/dl)	BIL T. (mg/dl)	BIL	
Group A: Normal Control	20.75 ± 3.84	14.50 ± 2.60	89.01 ± 12.36	0.31 ± 0.06	0.96 ± 0.32		
Group B: Stressed untreated	42.25 ± 3.47	26.75 ± 4.94	166.98 ± 34.52 a	1.09 ± 0.16 a	1.97 ± 0.30a		
Group C: Standard drug (2.9 mg/kg Magnesium Sulphate)	33.00 ± 5.67	11.50 ± 0.87	120.75 ± 2.84	0.62 ± 0.13	1.14 ± 0.18d		
Group D: 100mg/kg <i>Z. officinale</i>	2.25 ± 3.07	13.00 ± 2.12	119.37 ± 5.68	0.75 ± 0.17	1.05 ± 0.14 d		
Group E: 200mg/kg <i>Z. officinale</i>	19.75 ± 4.03	16.25 ± 2.93	111.78 ± 14.88	0.55 ± 0.09	0.99 ± 0.09 d		
Group F: 100mg/kg <i>A. melegueta</i>	24.75 ± 7.75	18.25 ± 4.48	77.97 ± 8.15 d	0.39 ± 0.04 d	1.56 ± 0.22		
Group G: 200mg/kg <i>melegueta</i>	38.75 ± 1.84	15.25 ± 1.44	119.37 ± 17.72	0.74 ± 0.06	0.95 ± 0.01 d		
Group H: 100mg/kg <i>officinale</i> and <i>A. melegueta</i>	30.00 ± 6.45	13.75 ± 1.89	107.64 ± 14.12	0.56 ± 0.14	1.05 ± 0.01 d		
Group I: 200mg/kg <i>Z. officinale</i> and <i>A. melegueta</i>	22.25 ± 7.43	13.50 ± 4.72	116.61 ± 6.70	0.52 ± 0.17	0.77 ± 0.07 d		

Note: a total of 45 wistar rats were used for this analysis

a=Significant increase($P < 0.05$) with respect to group A (Normal control); d =Significant decrease($P < 0.05$) with respect to group B (Stressed untreated)

RESULTS FOR WHITE BLOOD DIFFERENTIALS

The result showed a significant increase ($P < 0.05$) in the level of eosinophil and basophil levels of extract-treated groups compared to the untreated control while there was a significant decrease in the eosinophil level of the extract-treated group compared to the untreated control.

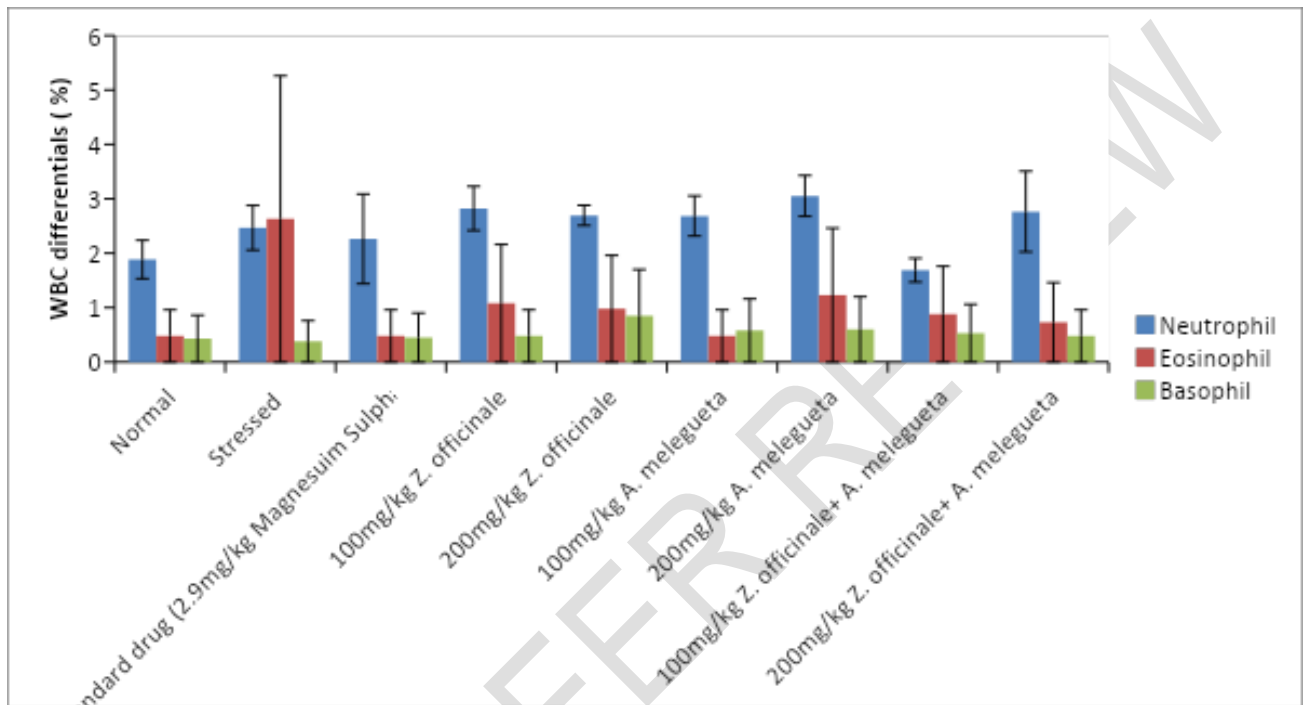


Figure 3: The effect of ethanol extract of *Z. officinale* and *A. melegueta* on white blood cells differentials of electric-shock stressed-induced rats before, during and after treatment period

RESULT FOR WHITE BLOOD CELL COUNT

The result showed a significant decrease ($P < 0.05$) in the white blood cell of extract-treated groups compared with the untreated control. However, co-administration of *Z. officinale* and *A. melegueta* at 200 mg/kg body weight showed an increase in the white blood cell compared with the untreated group.

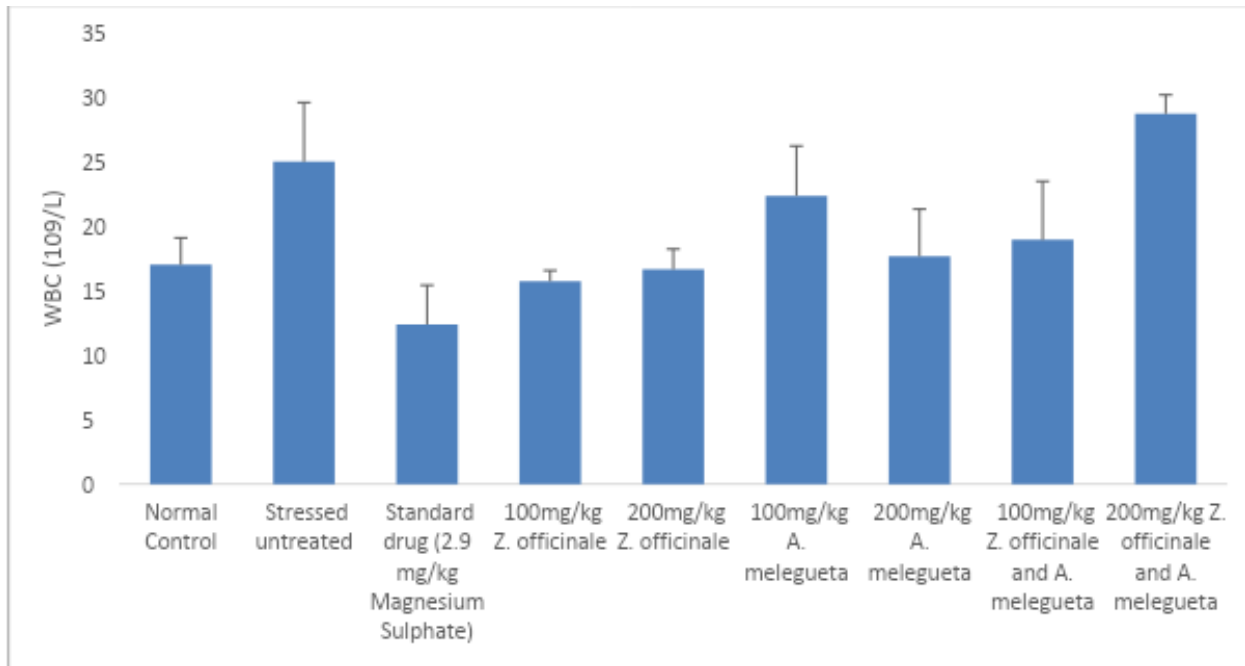


Figure 4: The effects of ethanol extract of *Z. officinale* and *A. melegueta* on white blood cell concentration of Electric-shock stress-induced rats.

RESULTS FOR PLATELET

Results on the effects of ethanol extract of *Z. officinale* and *A. melegueta* on Platelets concentration of electric-shock stress-induced rats are presented in Figure 5. Oral administration of 100 mg/kg body weight of *Z. officinale* and co-administration of *Z. officinale* and *A. melegueta* at 200 mg/kg significantly increases ($P < 0.05$) the platelet level of the treated groups when compared with the control group.

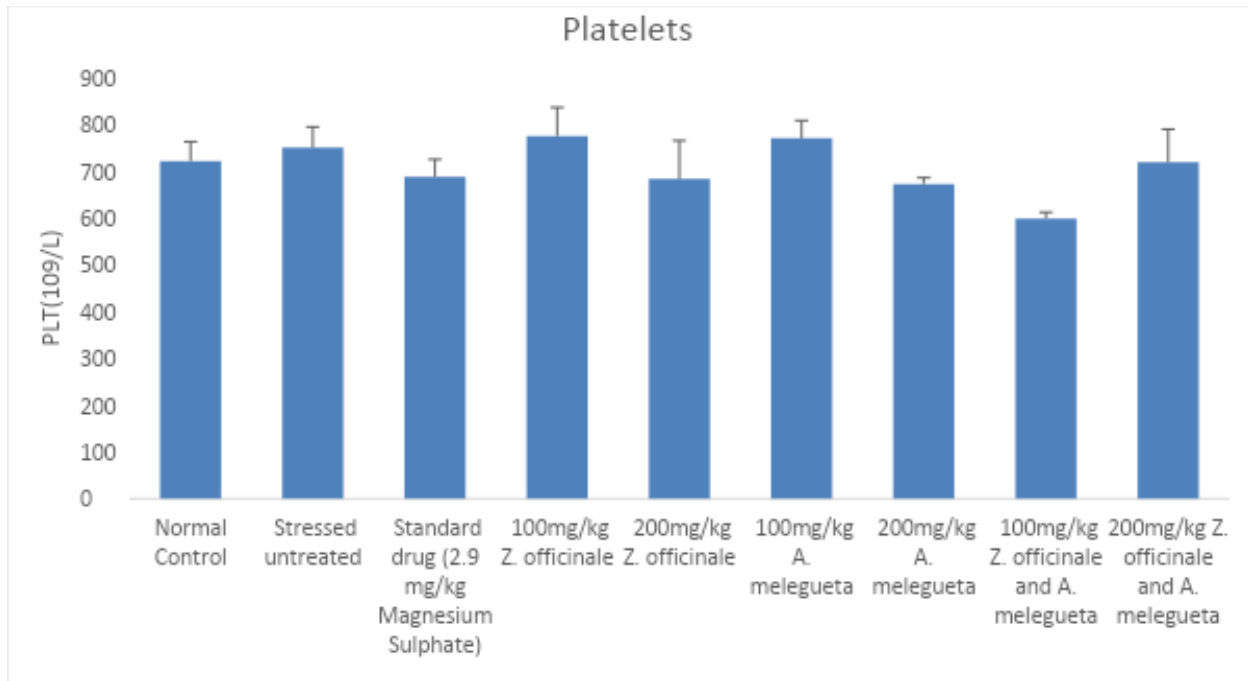


Figure 5: The effects of ethanol extract of *Z. officinale* and *A. melegueta* on Platelets concentration of electric-shock stress-induced rats.

RESULT FOR C- REACTIVE PROTEIN

Results on the effects of ethanol extract of *Z. officinale* and *A. melegueta* on C-reactive protein concentration of electric-shock stress-induced rats are presented in Figure 6. The result showed a significant decrease ($P < 0.05$) in extract-treated groups compared to the untreated group.

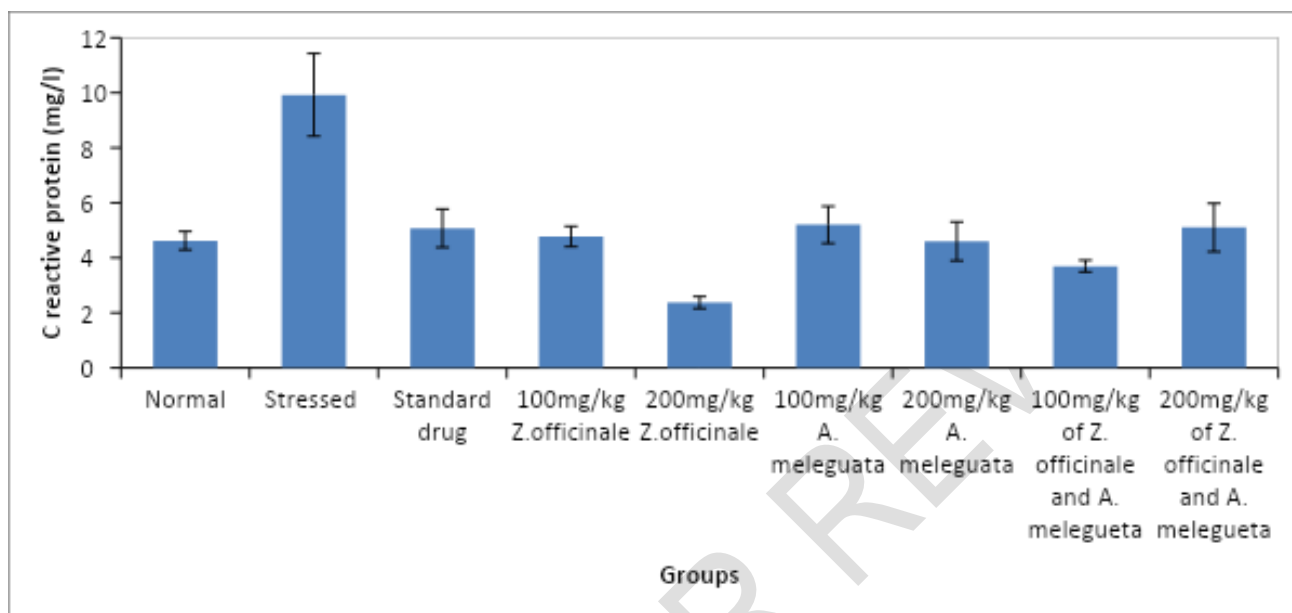


Figure 6: The effect of ethanol extract of *Z. officinale* and *A. melegueta* on c-reactive protein concentration of electric-shock stress-induced rats.

DISCUSSION

The results of the LD_{50} of the ethanol extract of *Zingiber officinale* rhizome and *Aframomum melegueta* obtained showed no death record thus the extracts could be said to be largely safe for consumption. The test animals showed that there was significant decrease ($p < 0.05$) in the cortisol levels of the extracts treated groups compared to the untreated control. It is worth noted that co-administration of *Z. officinale* and *A. melegueta* at 200 mg/kg body weight decreases ($p < 0.05$) the cortisol levels to nearly zero at week 1 of administration. This is an important observation since cortisol has been implicated in stressed conditions. Cortisol is a steroid hormone that is produced by the adrenal glands, which sit on top of each kidney and it is released into the bloodstream in a stressed state. This result is consistent with the findings of [1], who explained that when the homeostasis of the body is altered, the body releases hormones such as cortisol switches on the autonomic nervous system (ANS) which allows the body to adapt and respond to day-to-day activities. Since the induction of electric foot shock has been implicated in the pathogenesis of inflammatory response, the ability of these extracts to cause a decrease in the cortisol level supports its anti-inflammatory potentials. [22] made similar assertions. The result showing the effect of ethanol extract of *Z. officinale* and *A. melegueta* on Urea and Creatinine concentration in electric-shock stress induced rats is presented in table 1. As depicted in the figure above, there is a significant decrease ($p < 0.05$) in the urea and creatinine level of the extracts treated groups compared with the untreated control. This is consistent with the findings of [24]. Urea is a naturally occurring molecule that is produced by protein metabolism and found abundantly in mammalian urine. The normal range of urea nitrogen in blood or serum is 5 to 20 mg/dl. Accumulation of urea above this reference range has been implicated in several health conditions especially uremia. The levels of urea concentrations in the extract treated groups, as observed in this study implies that the administration of the extract does not pose any threat the proper physiological functions of the kidney. Similarly, normal reference range of creatinine have been reported to range between 0.7 to 1.3 mg/dl for adult males and 0.5 to 1.1 mg/dl for adult females. The observed creatinine level in this study falls within the reference range of apparently healthy patient. Creatinine is a waste product made by the muscles as part of regular, everyday activity. The kidneys filter creatinine from the blood and send it out of the body via urine. Hence, any impairment in the proper physiological functions of the kidneys could plausibly lead to accumulation of creatinine in the blood and consequently impair health. The observed activity of the enzymes in this study showed the protective effect of the plant extracts on the hepatocytes. [25] made similar assertion. The evaluation of hematological parameters is a useful information in determining the effect of foreign substances including plant extracts in vivo. They are used to ascertain any possible changes in the levels of biomolecules such as enzymes, metabolic

products, hematology, normal functioning and histopathology of the organs. White blood cells functions by mopping up foreign bodies by releasing macrophages [30]. In this study, the effect of extract of *Z. officinale* and *A. melegueta* on white blood cell and its differentials showed that there was a significant increase ($p < 0.05$) in the levels of the WBC in Figure 4 in the group treated with 200mg/kg of the combination of *Z. officinale* and *A. melegueta* gave a value of 28.72 ± 1.46 which is almost double of the value of stressed untreated 25.00 ± 4.59 and that of the normal group 17.01 ± 2.08 , although the dose-dependent value of *A. melegueta* at 100mg/kg gave a high value of 22.36 ± 3.86 but was suppressed at 200mg/kg, the combination of both extract at 200mg/kg proved to be more effective in producing more WBC in the process of treating stressed related inflammation. In figure 3 there was a significant increase ($p < 0.05$) of neutrophil in groups treated with 200mg/kg of both extracts it gave a value of 27.65 ± 7.41 which was higher than the stressed untreated group and those treated with standard drug, neutrophils are the first responders as they are swiftly recruited, constituting about 50% of all cell at the inflammation site [26]. The primary function of neutrophil at the site of inflammation includes compacting invading pathogens via various antimicrobial responses involving phagocytosis, therefore the groups treated with 100mg/kg and 200mg/kg of *Z. officinale*, 100mg/kg and 200mg/kg of *A. melegueta* were also effective in mobilizing the neutrophils but the most effective dose was that of 200mg/kg of the combination of the extracts. While Eosinophil levels were significantly reducing as see in figure 4, Eosinophils less commonly called acidophil are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates [26]. They form about 2 to 3% of WBC and are responsible for tissue damage, inflammation and allergy, from the result seen in figure 4, the eosinophils levels were not significantly increased, but rather there was a decrease as compared to the stressed untreated group, therefore this depicts that there was no presence of allergy or tissue damage, this is consistent with the findings of [27] as well as [28]. Neutrophil and monocytes as well as other hematological parameters are measurable indices of the blood, which can be used to evaluate hematopoietic function [28]. Neutrophils are important phagocytic cells normally elevated at early stage of stressed conditions [29]. Monocytes are known to be the largest type of leucocyte and can differentiate into macrophages and myeloid lineage dendritic cells, hence, they help in fighting bacteria, viruses, and other infections in the body, thereby influencing the process of adaptive immunity [30]. The platelets level is known to aggregate where there is wound healing or inflammation, from the results the groups treated with 200mg/kg of ginger and Alligator pepper combinations suppressed the platelets aggregation while 100mg/kg of ginger and Alligator pepper enhanced platelets aggregation comparing it to the groups treated with the standard drug, thereby enhancing the immune system. Thus the maximum efficacy of platelets is at 100mg/kg of both

extracts than the groups treated with 200mg/kg of both extracts and the standard drug. C-reactive protein (CRP) is a pentameric protein synthesized by the liver, whose level rises in response to inflammation. CRP is an acute-phase reactant protein that is primarily induced by the IL-6 action on the gene responsible for the transcription of CRP during the acute phase of an inflammatory/infectious process. A high level of CRP in the blood has been implicated in the pathogenesis of inflammation and inflammatory diseases. As depicted in this study (figure 6), induction of stress in the experimental animals significantly caused an increase ($p < 0.05$) in their serum CRP in the stressed untreated group, the CRP levels rose from 4.63 ± 0.33 to twice its normal level 9.93 ± 1.50 . However, the administration of extracts of *Z. officinale* and *A. melegueta* was able to mitigate this increase, bringing the serum CRP to nearly normal. This could plausibly be linked to the presence of bioactive compounds such as 6-gingerol and 6-shogaol in *Z. officinale*. 6-gingerol and 6-shogaol have been shown to have anti-inflammatory effect by inhibiting the production of inflammatory mediators, such as prostaglandin E₂, NO, inflammatory cytokines (TNF- α), interleukin-1 β (IL-1 β), and pro-inflammatory transcription factor (NF- κ B) [29]. [31] has earlier reported that root capsules of *Z. officinale* enhance analgesic and antioxidant efficacy of diclofenac sodium in experimental animals induced with acute inflammation. Similarly, [32] asserts that anti-inflammatory activities of plants are linked to their many bioactive compounds

CONCLUSION

The abundance of these nutritional fruits *Zingiberofficinale* and *Aframomummelegueta* makes it easily accessible and affordable by all; these plants have shown Anti-inflammatory abilities and have proven to be useful in the management and treatment of the silent pathway to many severe illnesses which is Stress. However, from the studies, a combination of the two extracts showed some favorable increase in some biochemical parameters such as the neutrophils and white blood cells, but the treatment with the extracts separately at 100mg/kg bw and 200mg/kg bw dose was also highly beneficial in reducing immunological indices such as the CRP comparing to the stressed untreated group, from time immemorial, medicinal plants especially *Zingiberofficinale* and *Aframomummelegueta* have been used in the treatment of different diseases. Hence from the results of this study, the administration of extract of *Zingiberofficinale* and *Aframomummelegueta* can be used to relief stressed conditions, tackle inflammation and consequently help to fight various pathological conditions.

ETHICAL APPROVAL

All experiments carried out in this research have been examined and approved by the ethics committee of NnamdiAzikiwe University, Awka, Nigeria in accordance with the Institutional Animal Care and Use policy in Research, Education and Testing. Issued reference number NAU/AREC/2023/00059.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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